NEUROSECRETION

IV. Localization of Neurosecretory Cells in the Central Nervous System of Limulus

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From an investigation, still under way, of the neuroglandular cells in the central nervous system of the horseshoe crab (*Limulus*), mention of which has been made in a previous review article (Scharrer and Scharrer, 1940), some data concerning the occurrence, localization, and numerical distribution of these cells are reported here. The publication of these data appears to be timely in view of the findings of Brown and Cunningham, reported in the preceding paper of this journal (1941). These authors demonstrate the presence of a chromatophorotropic principle within the nervous centers of *Limulus* and calculate the concentration of this substance in different, separately tested portions of the nervous system. They conclude that the chromatophorotropic principle is probably produced in definite groups of cells within the central nervous system. The agreement between the results of the physiological work of Brown and Cunningham and the morphological findings to be reported here suggests the possibility that in *Limulus* the neurosecretory cells are the source of the chromatophorotropic principle.

Material and Methods

Altogether forty adult male and female specimens of *Limulus polyphemus* were studied. Most of the material was preserved during the summer months of 1937, 1939, and 1940 at the Marine Biological Laboratory, Woods Hole.[1] Additional specimens were obtained alive from the New York Aquarium,[2] and were preserved for histological study at various intervals during the years 1938–1940. Furthermore, a few young specimens of *Limulus polyphemus* and two female *Limulus moluccanus*, one of them from Penang (Malay Peninsula) fixed in the summer

[1] For the use of research facilities during these periods the author is indebted to the Rockefeller Foundation and to the Rockefeller Institute for Medical Research, New York.

[2] I am obliged to Dr. C. W. Coates for his friendly assistance in obtaining this material.

of 1938, the other from the mangrove swamps at Chandipur (Orissa, India),[3] were included in this study.

In order to obtain comparable results, the same histological technique was used in all cases. The central nervous system, which in adult specimens is of considerable size, was carefully dissected out and was fixed in Zenker-formol. It was subsequently embedded in celloidin (nitrocellulose) and horizontal serial sections of 20 μ were stained with Foot's modification of Masson's trichrome method.

The various degrees of neurosecretory activity to be found in different parts of the central nervous system as well as in the different specimens of *Limulus* studied were estimated by counting in every section all cells containing secretory colloid. Thus undoubtedly a certain percentage of the cells was counted more than once because the vacuoles containing colloid are often large enough to appear in more than one section of 20 μ thickness. This is not to be considered an error of consequence, because this method of recording colloid whenever it appears in the sections, even if it belongs to the same cell, takes account of the volume of colloid as a whole rather than of the actual number of cells containing colloid inclusions. For purposes of comparing the secretory activity of different regions of the central nervous system this method appears to be satisfactory.

Observations

The Histological Appearance of the Neuroglandular Cells

It is not intended here to describe the cytological characteristics of cells considered as having a glandular function or to investigate the steps in which the transformation of a nerve cell into a gland cell takes place. In the present study the concern is only with such cells as are believed to represent the fully developed type of neurosecretory cell characteristic of *Limulus*. This cell type is fairly uniform and easily recognizable. The cells contain large masses of a colloidlike substance (Fig. 1) which appears homogeneous in sections treated in the manner described before. The substance stains green with the light green component of the Masson trichrome stain and seems to have physical properties not unlike those of the colloid of the thyroid gland (for corresponding parallels see also Hanström, 1941). This similarity is also suggested by vacuoles in the periphery of the colloid masses in the cells of *Limulus* which remind the observer of similar vacuoles seen in sections of thyroid colloid. Apparently this colloid mass pushes the nucleus and the cytoplasm aside and

[3] The efficient cooperation of Dr. Baini Prashad, Director of the Indian Museum, Calcutta, India, is gratefully acknowledged.

takes up a large space in the cell. These conspicuous colloid-carrying cells were counted.

Cells of this kind have also been found in the ganglia of cockroaches (Scharrer, 1941). However, in the cockroach they represent one of several kinds of neurosecretory cells, whereas in *Limulus* only this one type is encountered. There are in *Limulus* cells the appearance of which suggests that they represent phases preceding the fully developed "mature" neurosecretory cell but their description is not undertaken here, since their relation, if any, to the colloid-containing cells is still undetermined. From a histological point of view they certainly seem insignificant as a possible source of secretion material when compared with the

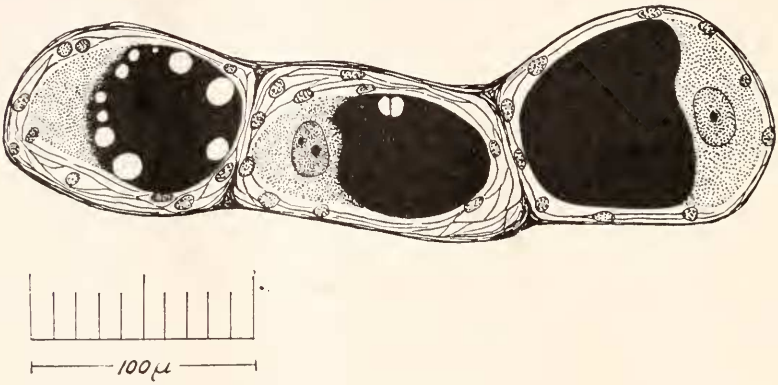

FIG. 1. Three neurosecretory cells, each in its capsule, from the circumesophageal ring of *Limulus polyphemus* with partly vacuolated colloid indicated in solid black. Zenker-formol, nitrocellulose, 20 μ, Masson.

cells containing the large masses of colloid. The contrast between the ordinary nerve cells, including those in which differences in stainability of the cytoplasm etc. suggest that they may be in a state of transformation into glandular elements, and the cells counted here as neurosecretory cells is always so definite that no doubt arises as to which cells should be included in the counts. *Limulus* is particularly favorable in this respect for the kind of investigation carried out here.

It should be repeated that the cells containing a homogeneous mass of colloid with varying numbers of marginal vacuoles are always observed when the histological technique described before is used. The fact that the appearance of the colloid is somewhat different after treatment by different methods is of no concern here where only the amount and distribution of the secretory elements are of interest.

The Localization of the Neuroglandular Cells within the Central Nervous System

The central nervous system of *Limulus* consists of the circumesophageal ring, situated in the cephalothorax, and the abdominal ganglia (Fig. 2). The ring contains the "brain" and a number of thoracic ganglia of the ventral cord, designated in Fig. 2 as Nos. 1–8, beginning with the cheliceral ganglia. There are, in addition, eight pairs of abdominal ganglia, only the first four or five of which are well defined, the remaining pairs being fused together (Patten and Redenbaugh, 1900).

With the exception of the corpora pedunculata which make up about three-fourths of the brain, all parts of the central nervous system of *Limulus* contain neurosecretory elements among the ordinary nerve cells. The distribution of the neurosecretory cells varies, however, with respect to the different ganglia and their total number shows the greatest variations from specimen to specimen.

A most "active" neurosecretory region is the posterior part of the circumesophageal ring, i.e. the area of the thoracic ganglia No. 6 and No. 7. Here the neuroglandular elements are found in clusters which may constitute a considerable proportion of the total mass of cells in the ganglion. These clusters of glandlike cells are arranged symmetrically with respect to the mid-sagittal plane (Fig. 3).

More anteriorly in the ring the neuroglandular cells become less and less frequent. They may even be absent in these portions, particularly in those specimens which, on the whole, show less neurosecretion than others. If present, neuroglandular cells in ganglia No. 1 to No. 5 appear single or in small groups of two or three. Their approximate number and position are the same on both the left and right side of the ring. Of all neurosecretory cells found in the circumesophageal ring only about one-tenth or less lie in the two anterior thirds, the majority being concentrated in the posterior third of the whole ring.

The abdominal ganglia, counted together, contain roughly on the average twice or three times as many neurosecretory elements as the corresponding ring. The numbers of neuroglandular cells counted in the circumesophageal ring and in the abdominal ganglia of four specimens are given here as examples:

Circumesophageal ring	117	206	264	516
Abdominal ganglia	340	442	861	1978

Thus as a rule and within limits, from the degree of neurosecretory activity in a given ring the activity in the abdominal ganglia of the same specimen can be predicted. The individual abdominal ganglia do not

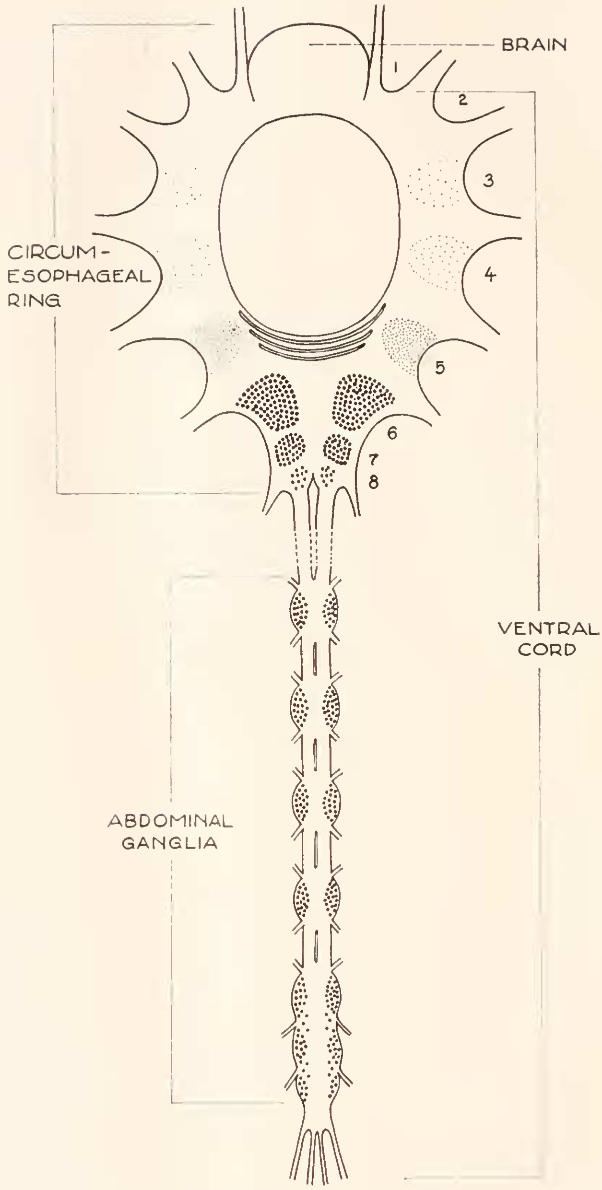

FIG. 2. Diagram of the central nervous system of *Limulus*. Numbers 1–8 are the ganglia of the circumesophageal ring. The areas where neurosecretory cells may be found are dotted. Coarser dots indicate a higher degree of neuroglandular activity than finer dots.

seem to differ significantly from each other in number of neurosecretory cells.

Individual Variations of Neurosecretory Activity

Whereas the central nervous system of a few young specimens of *Limulus polyphemus* (width of carapace from 5 to 8 cm.) does not contain neuroglandular cells, these cells are present in all adults studied thus far. This concerns animals from different geographical sources as well as from two different species. Furthermore, neurosecretion is found in both male and female *Limulus*.

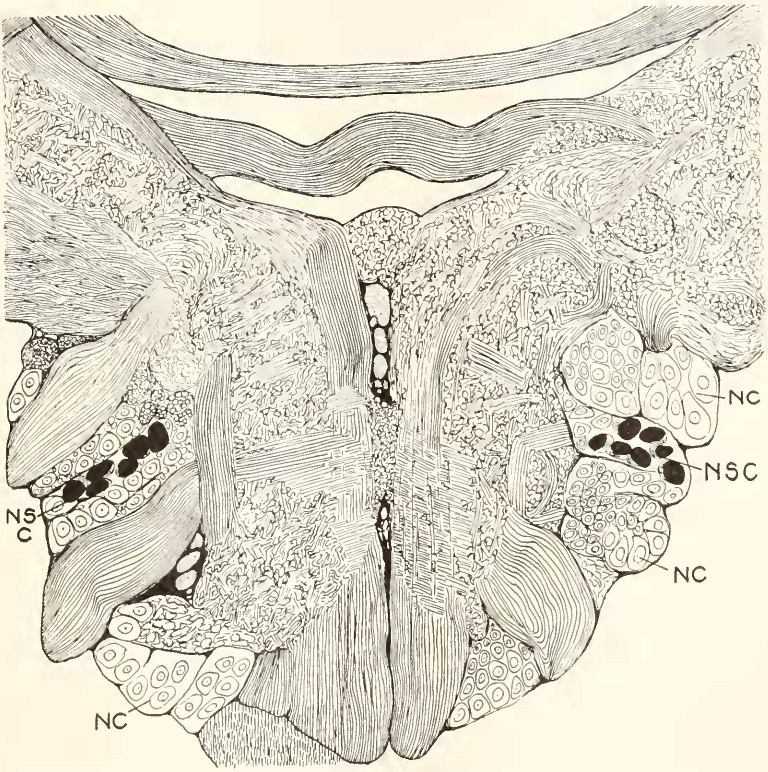

FIG. 3. Horizontal section through posterior part of the circumesophageal ring of *Limulus polyphemus*. *NSC*, neurosecretory cells; *NC*, nerve cells. The colloid of the neurosecretory cells which appears green with the histological technique used is indicated by solid black. Note symmetry of neurosecretory cell groups. Zenker-formol, nitrocellulose, 20 μ, Masson.

The degree of neurosecretory activity, however, varies considerably from specimen to specimen. In some, the entire central nervous system is found to contain only one or two neuroglandular cells, in others over a thousand such cells may be counted by the method described. The highest count thus far made, 2494, was in a large female. Between these extremes are such counts as 457, 648, 1125. These figures are given only to show the wide variation in view of which the errors necessarily involved in the calculation method employed here appear of minor importance.

The question may be asked next whether there exist any relations between the degree of neurosecretory activity and certain known factors. The following observations were made:

(1) A 24-hour cycle of secretion does not seem to exist. The histological appearance of neuroglandular cells is the same in different specimens fixed at various hours of the day.

(2) Neurosecretion in *Limulus* is not restricted to one time of the year, such as, for instance, the breeding season. None of the summer specimens contains more neuroglandular cells than the animals with the highest counts fixed in January, March, or November.

(3) The degree of neurosecretory activity in males and females is not essentially different. On the average, however, smaller numbers of neuroglandular cells are found in males, but this may be explained by the smaller average size of the male central nervous system.

(4) So far the only factor of some importance appears to be the age of the animals, as expressed by the size, i.e. the width of the carapace. As a rule, the larger specimens show a more active state of neurosecretion. The *Limulus* with the highest count of neurosecretory elements (almost 2500) was the largest specimen studied, a female of 32 cm. width which contained many and large eggs. On the other hand, none of five females under 23 cm. width showed more than about five secreting cells. Also among the males the largest specimen examined yielded the highest count but that does not exclude the fact that one or another small male may be encountered in a comparatively active state of neurosecretion. Thus, for instance, in the smallest male among a dozen studied, having a carapace-width of 16.5 cm., the relatively high number of 648 secreting cells was found. For comparison it may be noted that in two other male specimens these counts were made: carapace 19 cm., 5 cells; carapace 20 cm., 205 cells.

Although the existing evidence is not entirely conclusive, the extent to which nerve cells are engaged in secretory activity seems to run grossly parallel with age as expressed in the size of the animal. This fact has to be taken into account in attempts to influence experimentally the ratio of neuroglandular cells. Such experiments as have been carried out up to the present time were unsuccessful. In one extensive series extracts were made from the circumesophageal ring of the central nervous system of *Limulus* by grinding it with sand and sea water. The extracts from several, for instance five, different specimens of varying sizes and different sex, were pooled and injected into the body cavity of male and female specimens over varying periods of time. Approximate estimates were made of the total number of rings injected into each experimental animal. Thus, for instance, each of several animals got the equivalent of 17 rings over a period of one month. The counts made in such animals kept well within the limits of the normal variation:

♂	21 cm.	1073	♀	23 cm.	1
	21 "	617		23.5 "	22
	22 "	446		28 "	492

From the number of experiments done by the method described it can be safely concluded that sea water extracts from the neurosecretory cells of *Limulus* do not influence the number of cells engaged in secretion. It may be added also that no change in the histological appearance of these cells has been observed.

Similarly ineffectual were injections of pilocarpin. Of a 1 per cent solution in two specimens as much as 19 cc. were administered by means of two injections, both given on one day. Smaller amounts were injected in others. Again, the counts and the histological appearance of the cells gave no indication that pilocarpin acts on the neuroglandular cells in the concentrations used here which, in view of their general effect on the animals, may be considered as near the toxic ones.

Discussion

The data reported here on the occurrence and distribution of neurosecretory cells in the central nervous ganglia of *Limulus* correlate well with the findings of Brown and Cunningham (1941). The two authors describe a chromatophorotropic principle in extracts from all parts of the central nervous system of *Limulus polyphemus*. From the effect on crustacean chromatophores they conclude that the concentration of the active material varies with respect to different, separately tested parts of the central nervous system. The present histological study demonstrates the presence of nerve cells offering the picture of gland cells in all portions of the nervous system tested by Brown and Cunningham. The distribution of these elements in the nervous tissue varies in different regions, and this variation corresponds well with the distribution of the chromatophorotropic material found by these two authors. This correlation becomes particularly evident in the different portions of the circumesophageal ring. Its posterior third for which Brown and Cunningham report the greatest concentration of the active principle contains the majority of neurosecretory elements present in the ring. In the lateral sectors where the relative chromatophorotropic activity shows a considerable decrease, many fewer cells are found to contain secretory colloid. The anterior portion of the ring with relatively the lowest action on crustacean chromatophores contains glandular cells only occasionally. The connectives between the circumesophageal ring and the abdominal ganglia did not yield the chromatophorotropic principle; neither colloid nor neuroglandular cells are found in these connectives.

Considering that the estimate of the concentration of the chromatophorotropic principle in the nervous system of *Limulus* must necessarily be approximate, and that the cell counts may mean little in terms of function, the correlation between physiological and anatomical findings demonstrated here seems all that can be expected.

Within its obvious limitations this correlation consequently suggests that the neurosecretory cells in the central nervous system of *Limulus* may be considered as the source of the chromatophorotropic principle. If this proves to be correct the functional significance of neurosecretion assumes a new aspect. Thus far only one function has, with good evidence, been attributed to neuroglandular cells, namely the production of a hormone controlling molting in insects (Wigglesworth, 1940).

Summary

The occurrence, localization, and quantitative distribution of neurosecretory cells in the central nervous system of *Limulus* have been described. These anatomical findings are in good agreement with the physiological data of Brown and Cunningham (1941), who demonstrate the presence and distribution of a chromatophorotropic principle in the nervous system of this animal. Therefore, the neurosecretory cells of *Limulus* may be considered as the source of a substance influencing color change in crustaceans.

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